

What is Claimed is:

1. A recombinant slow-growing mycobacterium comprising at least one mycobacterial gene containing an unmarked mutation.
2. The recombinant slow-growing mycobacterium of Claim 1 wherein the mutant mycobacterial gene comprises a deletion, addition, substitution or point mutation.
3. The recombinant slow-growing mycobacterium of Claim 2 wherein the mycobacterial gene is a gene that encodes an enzyme essential in the biosynthetic pathway of a nutrient, structural component or an amino acid.
4. The recombinant slow-growing mycobacterium of Claim 3 wherein the mycobacterium is auxotrophic for lysine.
5. The recombinant slow-growing mycobacterium of Claim 4 wherein the mycobacterial gene is *lysA*.
6. The recombinant slow-growing mycobacterium of Claim 5 which is selected from the group of *M. bovis BCG*, *M. tuberculosis*, and *M. leprae*.
7. A method for preparing the recombinant slow-growing mycobacterium of Claim 1, comprising:
  - (a) introducing a vector into a slow-growing mycobacterium, said vector comprising a selectable marker, a counterselectable marker, and an unmarked mutant mycobacterial gene;
  - (b) selecting for primary recombinants incorporating the selectable marker;
  - (c) culturing the primary recombinants incorporating the selectable marker;
  - (d) selecting for secondary recombinants that have lost the counterselectable marker; and
  - (f) isolating the secondary recombinants comprising the desired unmarked mutant mycobacterial gene.
8. The method of Claim 7, wherein the vector is a suicide plasmid.

9. The method of Claim 8, wherein the selectable marker confers antibiotic resistance and the counterselectable marker is one of *rpsL*, *pyrF*, and *sacB*.

10. The method of Claim 9, wherein the counterselectable marker is *sacB*.

11. The method of Claim 7, wherein the recombinant slow-growing mycobacterium is auxotrophic for lysine.

12. The method of Claim 11, wherein the mycobacterial gene is *lysA*.

13. A vaccine that comprises (i) a recombinant slow-growing mycobacterium comprising at least one mycobacterial gene containing an unmarked mutation and (ii) a physiologically acceptable carrier.

14. The vaccine of Claim 13, wherein the unmarked mutation is a deletion, addition, substitution or point mutation.

15. The vaccine of Claim 14, wherein the slow-growing mycobacterium is auxotrophic for lysine.

16. The vaccine of Claim 15, wherein the gene is *lysA*.

17. The vaccine of Claim 16, wherein the slow-growing mycobacterium is selected from *M. bovis BCG*, *M. tuberculosis*, and *M. leprae*.

18. A method of treating or preventing tuberculosis in a subject comprising administering the vaccine of Claim 17 in an amount effective to treat or prevent tuberculosis in the subject.